ELSEVIER

FEMS Yeast Research 1 (2001) 177–185

FEMS
Yeast Research

www.fems-microbiology.org

Six new anamorphic ascomycetous yeasts near *Candida tanzawaensis*

Cletus P. Kurtzman *

Microbial Genomics and Bioprocessing Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, US Department of Agriculture, 1815 N. University Street, Peoria, IL 61604, USA

Received 26 March 2001; received in revised form 20 July 2001; accepted 2 August 2001

First published online 21 September 2001

Abstract

Six new species of the yeast genus *Candida* are described from their unique nucleotide sequences in the D1/D2 domain of 26S rDNA. Five of these species form a clade with *Candida tanzawaensis*, and the sixth is basal to this group. The new species and their sources of isolation are the following: *Candida ambrosiae* (type strain NRRL YB-1316, CBS 8844), from insect frass, rotted wood and mushroom fruiting bodies; *Candida canberraensis* (type strain NRRL YB-2417, CBS 8846), from soil; *Candida caryicola* (type strain NRRL YB-1499, CBS 8847), from a pignut hickory tree; *Candida prunicola* (type strain NRRL YB-869, CBS 8848), from exuded gum of a black cherry tree; *Candida pyralidae* (type strain NRRL Y-27085, CBS 5035), from insect frass; *Candida xylopsoci* (type strain NRRL Y-27066, CBS 6037), from insect frass. Published by Elsevier Science B.V. on behalf of the Federation of European Microbiological Societies.

Keywords: New *Candida* yeast species; Yeasts from insects; Molecular systematics; Ribosomal DNA

1. Introduction

Species-specific gene sequences are increasingly being used to identify yeasts. One such gene is the ca. 600-nucleotide D1/D2 domain of large subunit (26S) ribosomal DNA (rDNA), which resolves all but the most closely related species. Databases of D1/D2 sequences are now available for all currently recognized ascomycetous and basidiomycetous yeasts ([1,2], and subsequent GenBank entries), making the task of species identification relatively straightforward. In the course of molecular characterization of unidentified yeasts in the Agricultural Research Service (ARS) Culture Collection (NRRL), six new species of *Candida* were detected, and their descriptions are presented here. These species are of particular interest because they are closely related to *Candida tanzawaensis*, a species that previously had no known close relatives [1]. Consequently, this finding strengthens the belief that only a small number of extant yeast species are known and that molecular comparisons must be used if the full extent of yeast biodiversity is to be understood.

2. Materials and methods

2.1. Yeast strains and growth tests

Strains of the new species studied are listed in Table 1, and all are maintained in the Agricultural Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research, Peoria, IL, USA. The composition of culture media used in this study as well as procedures for fermentation, assimilation and other growth tests standard to yeast taxonomy are given by Yarrow [3].

2.2. rDNA sequencing and phylogenetic analysis

Methods for nuclear DNA isolation, amplification of the 26S rDNA D1/D2 domain by polymerase chain reaction (PCR), and sequencing with the ABI TaqDyeDeoxy Terminator Cycle sequencing kit/ABI Model 377 automated DNA sequencer (PE Biosystems, Inc., Foster City, CA, USA) were previously described [1]. All sequences reported are based on sequencing both DNA strands. Phylogenetic relationships were calculated using the maximum parsimony program of PAUP* 4.063a [4] with heuristic searches employing both simple and random sequence additions. Confidence limits for phylogenetic trees were estimated from bootstrap analyses (1000 repli-

* Tel.: (309) 681 6561; Fax: (309) 681 6672.
E-mail address: kurtzman@mail.ncaur.usda.gov (C.P. Kurtzman).

1567-1356/01/$20.00 Published by Elsevier Science B.V. on behalf of the Federation of European Microbiological Societies.
PII: S1567-1356(01)00035-6

Table 1
Strains of the new *Candida* species described

Species	Strain designation[a,b]		GenBank accession No. for 26S domain D1/D2 sequences	Source
	NRRL	CBS		
C. ambrosiae	YB-1316^T	8844	AY013716	Frass, insect tunnel, dead elm tree, Peoria, IL, USA.
	YB-1928	8845		Rotted log of unidentified tree, McCormicks State Park, Spencer, IN, USA.
	YB-1949			Unidentified pink coral fungus, McCormicks State Park, Spencer, IN, USA.
	YB-2289			Unidentified mushroom on a rotted log, Peoria, IL, USA.
	YB-2317			Frass, insect tunnel, sugar maple tree, Laconia, NH, USA.
	YB-2725			Frass, insect tunnel, oak tree, Matthiessen State Park, IL, USA.
C. canberraensis	YB-2417^T	8846	AY013718	Soil, hillside near Canberra, Australia.
C. caryicola	YB-1499^T	8847	AY013717	Pignut hickory tree, Peoria, IL, USA.
C. prunicola	YB-869^T	8848	AY013714	Exuded gum, black cherry tree, Peoria, IL, USA.
C. pyralidae	Y-27085^T	5035	AY013715	Frass from Pyralidae larvae, South Africa.
C. xylopsoci	Y-27066^T	6037	AY013719	Tunnel of *Xylopsocus capucinus* in *Celtis africana*, Natal, South Africa.

[a] 26S domain D1/D2 rDNA sequences were identical for all strains of *C. ambrosiae*.
[b] T, type strain; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, USA; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

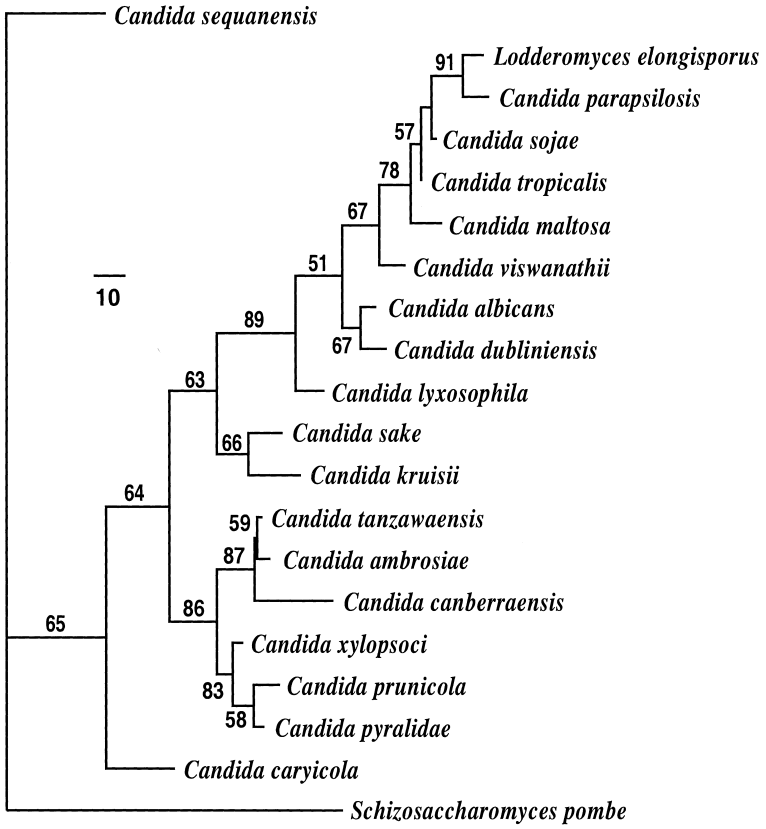

Fig. 1. One of two most parsimonious phylogenetic trees calculated from maximum parsimony analysis of 26S domain D1/D2 rDNA sequences depicting the relationships of the six new species of *Candida* with *C. tanzawaensis* and reference species in the neighboring *L. elongisporus*/*C. sake*/*C. sequanensis* clades. *Schizosaccharomyces pombe* was the outgroup species in the analysis. Branch lengths are proportional to nucleotide differences, as indicated by the bar, and numbers given at nodes are the percentage of frequencies with which a given branch appeared in 1000 bootstrap replications. Tree length = 426, consistency index = 0.627, retention index = 0.683, number of parsimony-informative characters = 114 of 601 total characters in the dataset. All sequences are from the type strains of the species shown. GenBank accession numbers for the new *Candida* species are given in Table 1; accession numbers for the other species were given by Kurtzman and Robnett [1].

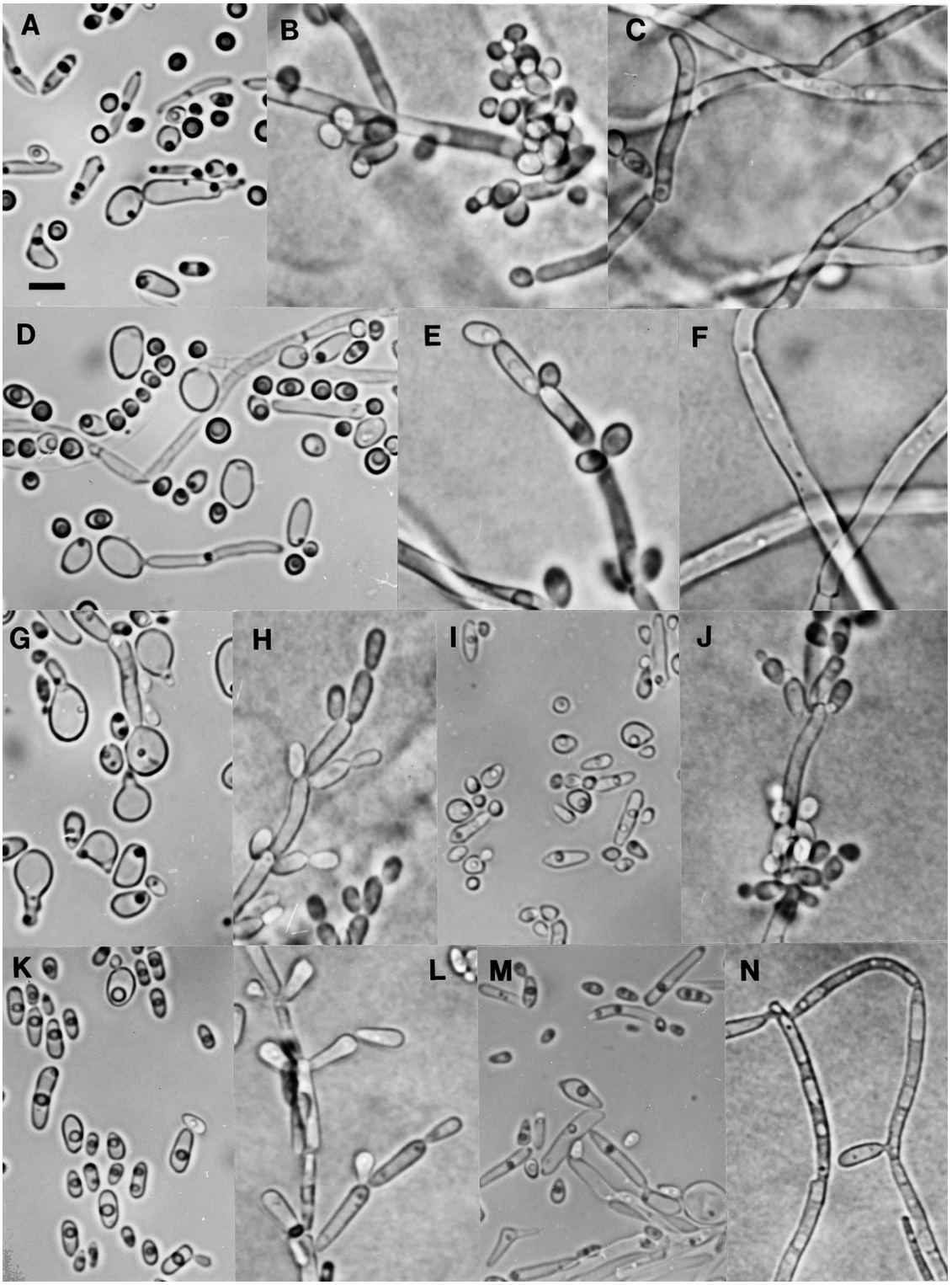

Fig. 2. New species of *Candida*. *C. ambrosiae* NRRL YB-1316. A: Budding cells. B: Pseudohyphae with blastoconidia. C: Pseudohyphal cells showing constrictions at the septa. *C. canberraensis* NRRL YB-2417. D: Budding cells. E: Pseudohyphae with blastoconidia. F: Pseudohyphae with prominent septa. *C. caryicola* NRRL YB-1499. G: Budding cells; inflated spherical cells present in most of the new species are common in this photograph and some show small denticles that bear blastoconidia. H: Pseudohyphae with blastoconidia. *C. prunicola* NRRL YB-869. I: Budding cells. J: Pseudohyphae with blastoconidia. *C. pyralidae* NRRL Y-27085. K: Budding cells. L: Pseudohyphae with elongate blastoconidia. *C. xylopsoci* NRRL Y-27066. M: Budding cells. N: Well-developed pseudohyphae showing only slight constrictions at the septa. Budding cells are from 5% malt extract agar, 3 d, 25°C; pseudohyphae are from Dalmau plate cultures on yeast morphology agar, 7 d, 25°C. Bar = 5 μm for all figures.

cations). All nucleotide sequences analyzed have been deposited with GenBank under the accession numbers given in Table 1 or those listed by Kurtzman and Robnett [1].

3. Results and discussion

3.1. Detection and description of the new species

The six new taxa to be described were recognized as novel species from their divergence in the variable 600-nucleotide D1/D2 domain of 26S rDNA following comparison with a database containing D1/D2 sequences from all currently recognized ascomycetous yeasts ([1], and subsequent entries in GenBank). Kurtzman and Robnett [1] demonstrated for ascomycetous yeasts that strains differing by more than 1% substitutions in this rDNA domain represent separate species. Fig. 1 shows the relationships of the proposed new species with their nearest neighbors among known species. The most closely related species pair is *Candida* sp. nov. NRRL YB-1316 and *C. tanzawaensis*, which differ from one another by 1.5% substitutions (9 of 601 nucleotides), whereas the most divergent pair is *Candida* sp. nov. NRRL YB-1499 and *Candida* sp. nov. NRRL YB-2417, which differ by 12.3% substitutions (74 of 601 nucleotides). In view of the genetic separation exhibited by the strains and the absence of ascosporulation, the following six new species are proposed for the genus *Candida*.

3.1.1. Latin diagnosis of Candida ambrosiae Kurtzman, sp. nov.

In agaro malti post dies 3 *ad* 25°C, *cellulae vegetativae globosae* (2.0–6.0 μm), *ellipsoideae aut elongatae* (1.9–4.5×3.0–25.0 μm), *singulae et binae. In agaro morphologico post dies* 7 *ad* 25°C, *incrementum fuscum pallidum, hebes, butyrosum; centrum colonia sublatum; margo glabro vel undulato. Pseudohyphae fiunt; hyphae verae non fiunt. Ascosporae non fiunt. Glucosum, sucrosum (infirme), maltosum (infirme, variabile) et trehalosum fermentantur. Galactosum, lactosum et raffinosum non fermentantur. Assimilantur glucosum, galactosum, sucrosum, maltosum, cellobiosum, trehalosum, melezitosum,* D-*xylosum,* L-*arabinosum,* D-*arabinosum (variabile),* D-*ribosum,* D-*glucosaminum, N-acetyl*-D-*glucosaminum, ethanolum, glycerolum, erythritolum, ribitolum,* D-*mannitolum,* D-*glucitolum, α-methyl*-D-*glucosidum, salicinum,* D-*gluconas,* 2-*keto*-D-*gluconas, acidum succinicum, acidum citricum, hexadecanum et cadaverinum. Non assimilantur* L-*sorbosum, lactosum, melibiosum, raffinosum, inulinum, amylum solubile,* L-*rhamnosum, methanolum, galactitolum,* 5-*keto*-D-*gluconas, saccharatas,* DL-*acidum lacticum, inositolum et potasii nitras. Amylum non formatur. Vitaminae externae ad crescentiam necessaria sunt. Crescit in medio* 10 μg ml^{-1} *cycloheximido addito, variabile in medio* 100 μg ml^{-1}. *Gelatinum non liquescit; pellicula fiunt (variabile). Augmentum fiunt in temperatura* 37°C. *Typus:* NRRL YB-1316 (CBS 8844) *designat stirpem typicam. Isolata a dejectus coleopterorum in Ulmus sp.,* Peoria, IL, USA. *Depositata in Collectione Culturarum* ARS (NRRL), Peoria, IL, USA.

3.1.2. Description of C. ambrosiae Kurtzman, sp. nov.

3.1.2.1. Growth on 5% malt extract agar. After 3 d at 25°C, yeast cells are spherical (2.0–6.0 μm) to ellipsoidal to elongate (1.9–4.5×3.0–25.0 μm), and single or in pairs (Fig. 2A). Elongated cells are straight or curved and may produce small denticles that bear blastoconidia. Some elongated cells form an inflated spherical tip cell that may also form denticles that give rise to blastoconidia. Budding is multilateral, but often occurs near the poles of the parent cell. There are usually 1–3 buds per cell. Colonies are white in color, dull to almost powdery, but with a butyrous texture.

3.1.2.2. Dalmau plate culture on yeast morphology agar. After 7 d at 25°C, growth under the coverglass shows abundant pseudohyphae that often bear clusters of blastoconidia (Fig. 2B). True hyphae were not seen, but some of the pseudohyphae show well-developed septations between cells (Fig. 2C). Aerobic growth is dull, white and almost powdery in appearance. Colonies are low with a central plateau and margins may be smooth to finely lobed. Texture ranges from butyrous to somewhat mycelial.

3.1.2.3. Fermentation, assimilation and other growth tests. Reactions are given in Table 2. Thin pellicles were occasionally formed on stationary liquid media.

3.1.2.4. Type. NRRL YB-1316 (CBS 8844) is preserved as a lyophilized preparation in the Agricultural Research Service Culture Collection (NRRL), Peoria, IL, USA. The strain was isolated in 1949 by L.J. Wickerham from frass in an insect tunnel in a dead elm tree (*Ulmus* sp.), Peoria, IL, USA.

3.1.2.5. Etymology. The species name *ambrosiae* refers to ambrosia beetles, the tunnels of which served as the source of the type strain as well as two of the other isolates of this species.

3.1.3. Latin diagnosis of Candida canberraensis Kurtzman, sp. nov.

In agaro malti post dies 3 *ad* 25°C, *cellulae vegetativae globosae* (2.0–4.5 μm), *ellipsoideae aut elongatae* (2.0–3.5×3.0–20.0 μm), *singulae et binae. In agaro morphologico post dies* 7 *ad* 25°C, *incrementum fuscum pallidum, hebes, butyrosum; centrum colonia sublatum; margo glabro vel undulato. Pseudohyphae fiunt; hyphae verae non fiunt. Ascosporae non fiunt. Glucosum, galactosum (infirme) et trehalosum fermentantur. Sucrosum, maltosum, lactosum*

Table 2
Physiological characteristics of the six new *Candida* species

Physiological test	Reaction of[a,b]					
	C. ambrosiae	*C. canberraensis*	*C. caryicola*	*C. prunicola*	*C. pyralidae*	*C. xylopsoci*
Fermentation						
Glucose	+	+	w	+	+	w
Galactose	−	w	−	−	−	−
Sucrose	w	−	−	−	−	−
Maltose	w/−	−	−	−	−	−
Lactose	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−
Trehalose	+	+	−	+	+	w
Assimilation						
Glucose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
L-Sorbose	−	+	−	+	+	−
Sucrose	+	+	+	−	−	−
Maltose	+	+	+	−	−	−
Cellobiose	+	+	−	−	−	+
Trehalose	+	+	+	+	−	−
Lactose	−	−	−	−	−	−
Melibiose	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−
Melezitose	+	+	+	−	−	−
Inulin	−	−	−	−	−	−
Soluble starch	−	−	−	−	−	−
D-Xylose	+	+	−	+	+	+
L-Arabinose	+	−	−	−	+	−
D-Arabinose	v	−	+	−	−	−
D-Ribose	+	+	−	−	+	−
L-Rhamnose	−	−	−	−	−	−
D-Glucosamine	+	+	+	+	+	+
N-Acetyl-D-glucosamine	+	+	+	+	+	+
Methanol	−	−	−	−	−	−
Ethanol	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+
Erythritol	+	+	−	−	−	−
Ribitol	+	+	+	+	+	+
Galactitol	−	−	−	−	−	−
D-Mannitol	+	+	+	+	+	+
D-Glucitol	+	+	+	+	+	+
α-Methyl-D-glucoside	+	+	+	−	−	−
Salicin	+	+	−	−	−	+
D-Gluconate	+	+	+	+	+	+
2-Keto-D-gluconate	+	+	+	+	+	+
5-Keto-D-gluconate	−	−	−	−	−	−
Saccharate	−	−	−	−	−	−
DL-Lactate	−	−	−	−	−	−
Succinate	+	+	+	−	+	+
Citrate	+	+	+	+	+	+
Inositol	−	−	−	−	−	−
Hexadecane	+	+	+	−	+	+
Nitrate	−	−	−	−	−	−
Additional growth tests						
Vitamin-free medium	−	−	−	−	+	+
Cadaverine	+	+	+	+	+	+
10% NaCl/5% glucose	+	+	+	+	+	+
Starch formation	−	−	−	−	−	−
Gelatin liquefaction	−	−	−	−	−	−
Cycloheximide, 10 μg ml^{-1}	+	+	−	−	+	−
Cycloheximide, 100 μg ml^{-1}	v	−	−	−	−	−
Growth at 37°C	+	−	−	+	+	+

[a] −, negative; +, positive; w, weak; w/−, weak or negative; v, strain variable, i.e. + or −.
[b] Reactions for *C. ambrosiae* are from the six strains listed in Table 1. Reactions for the other species are based on the type strains.

et raffinosum non fermentantur. Assimilantur glucosum, galactosum, L-*sorbosum, sucrosum, maltosum, cellobiosum, trehalosum, melezitosum,* D-*xylosum,* D-*ribosum,* D-*glucosaminum, N-acetyl*-D-*glucosaminum, ethanolum, glycerolum, erythritolum, ribitolum,* D-*mannitolum,* D-*glucitolum, α-methyl*-D-*glucosidum, salicinum,* D-*gluconas,* 2-*keto*-D-*gluconas, acidum succinicum, acidum citricum, hexadecanum et cadaverinum. Non assimilantur lactosum, melibiosum, raffinosum, inulinum, amylum solubile,* L-*arabinosum,* D-*arabinosum,* L-*rhamnosum, methanolum, galactitolum,* 5-*keto*-D-*gluconas, saccharatas,* DL-*acidum lacticum, inositolum et potasii nitras. Amylum non formatur. Vitaminae externae ad crescentiam necessaria sunt. Crescit in medio* 10 μg ml^{-1} *cycloheximido addito, non in* 100 μg ml^{-1}. *Gelatinum non liquescit; pellicula fiunt. Augmentum non fiunt in temperatura* 37°C. *Typus:* NRRL YB-2417 (CBS 8846) *designat stirpem typicam. Isolata a terra ex* Canberra, Australia. *Depositata in Collectione Culturarum* ARS (NRRL), Peoria, IL, USA.

3.1.4. Description of C. canberraensis Kurtzman, sp. nov.

3.1.4.1. Growth on 5% malt extract agar. After 3 d at 25°C, yeast cells range from spherical (2.0–4.5 μm) to ellipsoidal to elongate (2.0–3.5×3.0–20.0 μm), and are single or in pairs. Budding is multilateral but predominantly near the poles of the cells (Fig. 2D). Elongated cells may be straight or curved and some form small denticles that bear blastoconidia. Not uncommonly, elongated cells form an inflated spherical tip cell that may also form denticles with blastoconidia. Colonies are white, dull to almost powdery and butyrous in texture.

3.1.4.2. Dalmau plate culture on yeast morphology agar. After 7 d at 25°C, growth under the coverglass is composed of abundant pseudohyphae with blastoconidia (Fig. 2E). True hyphae were not found, but some of the pseudohyphae exhibit well-defined septa (Fig. 2F). Aerobic growth is dull, white and butyrous to mycelial. Colonies are low convex with a slightly raised center and with margins that are entire or infrequently lobed.

3.1.4.3. Fermentation, assimilation and other growth tests. Reactions are given in Table 2. Moderately thick pellicles are formed on stationary liquid media.

3.1.4.4. Type. NRRL YB-2417 (CBS 8846) is preserved as a lyophilized preparation in the Agricultural Research Service Culture Collection (NRRL), Peoria, IL, USA. The strain was isolated in 1950 by L.J. Wickerham from a soil sample collected on a hillside near Canberra, Australia.

3.1.4.5. Etymology. The species name *canberraensis* refers to the city of Canberra, Australia, the location of the soil sample yielding the type strain of this species.

3.1.5. Latin diagnosis of Candida caryicola Kurtzman, sp. nov.

In agaro malti post dies 3 *ad* 25°C, *cellulae vegetativae globosae* (2.0–6.0 μm), *ellipsoideae aut elongatae* (2.0–5.0×3.1–25.0 μm), *singulae et binae. In agaro morphologico post dies* 7 *ad* 25°C, *incrementum fuscum pallidum, hebes, butyrosum; centrum colonia sublatum; margo glabro vel denticulatus. Pseudohyphae fiunt; hyphae verae non fiunt. Ascosporae non fiunt. Glucosum (infirme) fermentatur. Galactosum, sucrosum, maltosum, lactosum, raffinosum et trehalosum non fermentantur. Assimilantur glucosum, galactosum, sucrosum, maltosum, trehalosum, melezitosum,* D-*arabinosum,* D-*glucosaminum, N-acetyl*-D-*glucosaminum, ethanolum, glycerolum, ribitolum,* D-*mannitolum,* D-*glucitolum, α-methyl*-D-*glucosidum,* D-*gluconas,* 2-*keto*-D-*gluconas, acidum succinicum, acidum citricum, hexadecanum et cadaverinum. Non assimilantur* L-*sorbosum, cellobiosum, lactosum, melibiosum, raffinosum, inulinum, amylum solubile,* D-*xylosum,* L-*arabinosum,* D-*ribosum,* L-*rhamnosum, methanolum, erythritolum, galactitolum, salicinum,* 5-*keto*-D-*gluconas, saccharatas,* DL-*acidum lacticum, inositolum et potasii nitras. Amylum non formatur. Vitaminae externae ad crescentiam necessaria sunt. Non crescit in medio* 10 μg ml^{-1} *cycloheximido addito. Gelatinum non liquescit; pellicula fiunt. Augmentum non fiunt in temperatura* 37°C. *Typus:* NRRL YB-1499 (CBS 8847) *designat stirpem typicam. Isolata a nucis ex Carya glabra,* Peoria, IL, USA *Depositata in Collectione Culturarum* ARS (NRRL), Peoria, IL, USA.

3.1.6. Description of C. caryicola Kurtzman, sp. nov.

3.1.6.1. Growth on 5% malt extract agar. After 3 d at 25°C, yeast cells are spherical (2.0–6.0 μm) to ellipsoidal to elongate (2.0–5.0×3.1–25.0 μm), and single or in pairs. Budding is multilateral with 1–3 buds per cell that generally form near the poles of the cells. Longer cells are straight or curved, and produce small denticles that give rise to blastoconidia. Some of the longer cells divide forming a globose inflated terminal cell that often detaches. These globose cells may form rachis-like outgrowths that produce blastoconidia (Fig. 2G). Colonies are white with a dull, almost powdery surface. The texture of the colony is butyrous.

3.1.6.2. Dalmau plate culture on yeast morphology agar. After 7 d at 25°C, growth under the coverglass shows moderately branched pseudohyphae with blastoconidia (Fig. 2H). True hyphae are absent, but occasional pseudohyphae show distinct septa much like the preceding species. Aerobic growth is dull, white and colonies are low with a crateriform center. Margins are entire or with small

denticulate outgrowths. The texture of the colony is butyrous to somewhat mycelial.

3.1.6.3. Fermentation, assimilation and other growth tests. Reactions are given in Table 2. Moderately thick, climbing pellicles are formed on stationary liquid media.

3.1.6.4. Type. NRRL YB-1499 (CBS 8847) is preserved as a lyophilized preparation in the Agricultural Research Service Culture Collection (NRRL), Peoria, IL, USA. The strain was isolated in 1950 by L.J. Wickerham, apparently from a nut pod, of a pignut hickory tree (*Carya glabra* (Mill.) Sweet) growing in Peoria, IL, USA.

3.1.6.5. Etymology. The species name *caryicola* refers to the genus name for hickory (*Carya*), the source of the type strain.

3.1.7. Latin diagnosis of Candida prunicola Kurtzman, sp. nov.

In agaro malti post dies 3 *ad* 25°C*, cellulae vegetativae globosae* (2.1–4.0 μm)*, ellipsoideae aut elongatae* (1.4–3.5×2.0–17.0 μm)*, singulae, binae et fasciculatae. In agaro morphologico post dies* 7 *ad* 25°C*, incrementum fuscum pallidum, hebes, butyrosum; centrum colonia sublatum; margo glabro. Pseudohyphae fiunt; hyphae verae non fiunt. Ascosporae non fiunt. Glucosum et trehalosum fermentantur. Galactosum, sucrosum, maltosum, lactosum et raffinosum non fermentantur. Assimilantur glucosum, galactosum,* L-*sorbosum, trehalosum,* D-*xylosum,* D-*glucosaminum, N-acetyl-*D-*glucosaminum, ethanolum, glycerolum, ribitolum,* D-*mannitolum,* D-*glucitolum,* D-*gluconas,* 2-*keto-*D-*gluconas, acidum citricum et cadaverinum. Non assimilantur sucrosum, maltosum, cellobiosum, lactosum, melibiosum, raffinosum, melezitosum, inulinum, amylum solubile,* L-*arabinosum,* D-*arabinosum,* D-*ribosum,* L-*rhamnosum, methanolum, erythritolum, galactitolum, α-methyl-*D-*glucosidum, salicinum,* 5-*keto-*D-*gluconas, saccharatas,* DL-*acidum lacticum, acidum succinicum, inositolum, hexadecanum et potasii nitras. Amylum non formatur. Vitaminae externae ad crescentiam necessaria sunt. Non crescit in medio* 10 μg ml^{-1} *cycloheximido addito. Gelatinum non liquescit; pellicula non fiunt. Augmentum fiunt in temperatura* 37°C*. Typus:* NRRL YB-869 (CBS 8848) *designat stirpem typicam. Isolata a gummi ex Prunus serotina,* Peoria, IL, USA. *Depositata in Collectione Culturarum* ARS (NRRL), Peoria, IL, USA.

3.1.8. Description of C. prunicola Kurtzman, sp. nov.

3.1.8.1. Growth on 5% malt extract agar. After 3 d at 25°C, yeast cells are spherical (2.1–4.0 μm) to ellipsoidal (1.4–3.5×2.0–7.5 μm) to elongate (2.0–2.5×6.0–17.0 μm), and single, in pairs or occasionally in small clusters (Fig. 2I). Budding is multilateral with 1–3 buds per cell, usually near the poles of the cell. Elongate cells often form short denticles that produce blastoconidia. Occasional elongated cells form a globose inflated terminal cell. Colonies are white, dull, powdery, and butyrous in texture.

3.1.8.2. Dalmau plate culture on yeast morphology agar. After 7 d at 25°C, growth under the coverglass shows sparingly branched pseudohyphae with clusters of blastoconidia at the nodes (Fig. 2J). True hyphae were not produced. Aerobic growth is dull, tannish-white and butyrous in texture. Colonies are low with an elevated center showing pustule-like outgrowths; margins are smooth, but may be interrupted by pseudohyphal outgrowths.

3.1.8.3. Fermentation, assimilation and other growth tests. Reactions are given in Table 2. Pellicles do not form on stationary liquid media, but thin films of growth are present.

3.1.8.4. Type. NRRL YB-869 (CBS 8848) is preserved as a lyophilized preparation in the Agricultural Research Service Culture Collection (NRRL), Peoria, IL, USA. The strain was isolated in 1947 by L.J. Wickerham from gum exuded by a black cherry tree (*Prunus serotina* Ehrh.) growing in Peoria, IL, USA.

3.1.8.5. Etymology. The species name *prunicola* refers to the genus name for black cherry (*Prunus*), the source of the type strain.

3.1.9. Latin diagnosis of Candida pyralidae Kurtzman, sp. nov.

In agaro malti post dies 3 *ad* 25°C*, cellulae vegetativae globosae* (2.7–6.0 μm)*, ellipsoideae aut elongatae* (2.1–5.0×3.0–17.0 μm)*, singulae et binae. In agaro morphologico post dies* 7 *ad* 25°C*, incrementum fuscum pallidum, hebes, butyrosum; centrum colonia sublatum; margo undulato. Pseudohyphae fiunt; hyphae verae non fiunt. Ascosporae non fiunt. Glucosum et trehalosum fermentantur. Galactosum, sucrosum, maltosum, lactosum et raffinosum non fermentantur. Assimilantur glucosum, galactosum,* L-*sorbosum,* D-*xylosum,* L-*arabinosum,* D-*ribosum,* D-*glucosaminum, N-acetyl-*D-*glucosaminum, ethanolum, glycerolum, ribitolum,* D-*mannitolum,* D-*glucitolum,* D-*gluconas,* 2-*keto-*D-*gluconas, acidum succinicum, acidum citricum, hexadecanum et cadaverinum. Non assimilantur sucrosum, maltosum, cellobiosum, trehalosum, lactosum, melibiosum, raffinosum, melezitosum, inulinum, amylum solubile,* D-*arabinosum,* L-*rhamnosum, methanolum, erythritolum, galactitolum, α-methyl-*D-*glucosidum, salicinum,* 5-*keto-*D-*gluconas, saccharatas,* DL-*acidum lacticum, inositolum et potasii nitras. Amylum non formatur. Vitaminae externae ad crescentiam necessaria non sunt. Crescit in medio* 10 μg ml^{-1} *cycloheximido addito, non in* 100 μg ml^{-1}*. Gelatinum non liquescit; pellicula fiunt (variabile). Augmentum fiunt in*

temperatura 37°C. *Typus:* NRRL Y-27085 (CBS 5035) *designat stirpem typicam. Isolata a dejectus coleopterorum (Pyralidae) in arbor, Africa australis. Depositata in Collectione Culturarum* ARS (NRRL), Peoria, IL, USA.

3.1.10. Description of C. pyralidae Kurtzman, sp. nov.

3.1.10.1. Growth on 5% malt extract agar. After 3 d at 25°C, yeast cells are spherical (2.7–6.0 μm) to elongate (2.1–5.0×3.0–17.0 μm), and single or in pairs (Fig. 2K). Budding is multilateral with 1–3 buds per cell, primarily at the poles of the cells. Denticulate cells and inflated spherical cells, common to the preceding species, appear absent in *C. pyralidae*. Colonies are tannish-white, dull and butyrous in texture.

3.1.10.2. Dalmau plate culture on yeast morphology agar. After 7 d at 25°C, growth under the coverglass is quite restricted with sparse pseudohyphae and limited production of blastoconidia (Fig. 2L). True hyphae were not produced. Aerobic growth is dull, tannish-white and butyrous in texture. Colonies are very low with a small, slightly raised central plateau. Margins are irregularly lobed.

3.1.10.3. Fermentation, assimilation and other growth tests. Reactions are given in Table 2. Thin pellicles occasionally form on stationary liquid media.

3.1.10.4. Type. NRRL Y-27085 (CBS 5035) is preserved as a lyophilized preparation in the Agricultural Research Service Culture Collection (NRRL), Peoria, IL, USA. The strain was deposited in the CBS Yeast Collection in 1961 by J.P. van der Walt as an unidentified strain and had been isolated from frass of Pyralidae larvae in an unspecified tree in South Africa.

3.1.10.5. Etymology. The species name *pyralidae* refers to Pyralidae larvae, the source of the frass from which the type strain was isolated.

3.1.11. Latin diagnosis of Candida xylopsoci Kurtzman, sp. nov.

In agaro malti post dies 3 *ad* 25°C*, cellulae vegetativae globosae* (2.5–7.0 μm)*, ellipsoideae aut elongatae* (1.5–7.0×2.1–21.0 μm)*, singulae et binae. In agaro morphologico post dies* 7 *ad* 25°C*, incrementum fuscum pallidum, hebes, butyrosum; centrum colonia sublatum; margo undulato. Pseudohyphae fiunt; hyphae verae non fiunt. Ascosporae non fiunt. Glucosum (infirme) et trehalosum (infirme) fermentantur. Galactosum, sucrosum, maltosum, lactosum et raffinosum non fermentantur. Assimilantur glucosum, galactosum, cellobiosum,* D*-xylosum,* D*-glucosaminum, N-acetyl-*D*-glucosaminum, ethanolum, glycerolum, ribitolum,* D*-mannitolum,* D*-glucitolum, salicinum,* D*-gluconas, 2-keto-*D*-gluconas, acidum succinicum, acidum citricum, hexadecanum et cadaverinum. Non assimilantur* L*-sorbosum, sucrosum, maltosum, trehalosum, lactosum, melibiosum, raffinosum, melezitosum, inulinum, amylum solubile,* L*-arabinosum,* D*-arabinosum,* D*-ribosum,* L*-rhamnosum, methanolum, erythritolum, galactitolum, α-methyl-*D*-glucosidum, 5-keto-*D*-gluconas, saccharatas,* DL*-acidum lacticum, inositolum et potasii nitras. Amylum non formatur. Vitaminae externae ad crescentiam necessaria non sunt. Non crescit in medio* 10 μg ml^{-1} *cycloheximido addito. Gelatinum non liquescit; pellicula fiunt. Augmentum fiunt in temperatura* 37°C*. Typus:* NRRL Y-27066 (CBS 6037) *designat stirpem typicam. Isolata a dejectus Xylopsocus capucinus in Celtis africana, Natal, Africa australis. Depositata in Collectione Culturarum* ARS (NRRL), Peoria, IL, USA.

3.1.12. Description of C. xylopsoci Kurtzman, sp. nov.

3.1.12.1. Growth on 5% malt extract agar. After 3 d at 25°C, yeast cells are spherical (2.5–7.0 μm) to elongate (1.5–7.0×2.1–21.0 μm), and single or in pairs (Fig. 2M). Budding is multilateral with 1–2 buds per cell, usually formed near the poles of the cell. Cells occasionally form small denticles that bear blastoconidia. Cells with inflated spherical tip cells are common. Colonies are tannish-white, dull, butyrous, but with pseudomycelial margins.

3.1.12.2. Dalmau plate culture on yeast morphology agar. After 7 d at 25°C, growth under the coverglass shows abundant branched pseudohyphae, but few blastoconidia. True hyphae were not observed, but many of the pseudohyphal filaments have robust sparingly constricted septa (Fig. 2N). Aerobic growth is dull, tannish-white, and with a texture that ranges from butyrous to mycelial. Colonies are low convex with finely convoluted margins.

3.1.12.3. Fermentation, assimilation and other growth tests. Reactions are given in Table 2. Incomplete, moderately thick pellicles form on stationary liquid media.

3.1.12.4. Type. NRRL Y-27066 (CBS 6037) is preserved as a lyophilized preparation in the Agricultural Research Service Culture Collection (NRRL), Peoria, IL, USA. The strain was deposited in the CBS Yeast Collection in 1969, and had been isolated by D.B. Scott from the tunnel of *Xylopsocus capacinus* in *Celtis africana* growing in Natal, South Africa.

3.1.12.5. Etymology. The species name *xylopsoci* is derived from the genus name (*Xylopsocus*) of the beetle larva with which the type strain was associated.

3.2. Examination of the new Candida species for an ascosporic state

Individual strains of each of the six species, as well as a mixture of all six strains of *C. ambrosiae*, were placed on

YM, 5% malt extract, RG, and McClary's acetate agars and incubated at 15 and 25°C. Strains incubated at 25°C were examined weekly for 2 months and strains placed at 15°C were examined at 10–14 day intervals for 5 months. None of the individual strains, nor the mixture of *C. ambrosiae* strains, showed either conjugation or ascosporulation.

3.3. Phenotypic separation of the new species

As seen from Table 2, there are sufficient differences in physiological reactions to allow separation of the six new *Candida* species from one another, and from *C. tanzawaensis* based on the data reported by Meyer et al. [5]. However, physiological tests would not have clearly resolved these species from currently described taxa. For example, the fermentation and assimilation pattern of *C. ambrosiae* is essentially identical to that of *Candida atmosphaerica* [5], and the two species differ primarily on presence or absence of growth at 37°C. The other five new *Candida* species are physiologically nearly indistinguishable from a number of earlier described species [6], further emphasizing the need to use molecular comparisons for strain identification.

3.4. Phylogenetic placement of the new species

Phylogenetic analysis of D1/D2 domain sequences from all currently accepted anamorphic and teleomorphic ascomycetous yeasts has shown that a substantial number of species have no close neighbors [1]. Among these isolated species were *C. tanzawaensis* and *Candida sequanensis*, which show only weak affinities with each other as well as with the neighboring *Lodderomyces elongisporus* and *Candida sake* clades. One of the questions coming from that work was whether these species are genetically isolated because they are restricted to particular habitats, or because additional members of their clade either have not been isolated or have not been recognized as related.

Five of the new species described here form a clade with *C. tanzawaensis*, a species known from a single strain isolated from a moss plant growing on Mt. Tanzawa in Japan [7]. The sixth new species, *C. caryicola*, is basal to the *C. tanzawaensis* clade and only weakly associated with it (Fig. 1). The present molecular comparison further demonstrates that only a small number of extant yeast species have been recognized. Species of the *C. tanzawaensis* clade are widely distributed geographically (Japan, Australia, South Africa, USA), which suggests that many more species of this clade await discovery. Because a significant number of the isolates are from frass of beetle larvae, wood boring insects might represent an important source of species from the *C. tanzawaensis* clade.

Acknowledgements

Christie J. Robnett is gratefully acknowledged for determining the D1/D2 domain sequences for the new species, Larry W. Tjarks for skillful operation of the nucleic acid sequencer, and Eleanor Basehoar-Powers for conducting fermentation and assimilation tests. The mention of firm names or trade products does not imply that they are endorsed or recommended by the US Department of Agriculture over other firms or similar products not mentioned.

References

[1] Kurtzman, C.P. and Robnett, C.J. (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek 73, 331–371.

[2] Fell, J.W., Boekhout, T., Fonseca, A., Scorzetti, G. and Statzell-Tallman, A. (2000) Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. Int. J. Syst. Evol. Microbiol. 50, 1351–1371.

[3] Yarrow, D. (1998) Methods for the isolation, maintenance and identification of yeasts. In: The Yeasts, a Taxonomic Study, 4th edn. (Kurtzman, C.P. and Fell, J.W., Eds.), pp. 77–100. Elsevier Science B.V., Amsterdam, The Netherlands.

[4] Swofford, D.L. (1998) PAUP* 4.0: Phylogenetic Analysis Using Parsimony. Sinauer Associates, Sunderland, MA.

[5] Meyer, S.A., Payne R.W. and Yarrow, D. (1998) *Candida* Berkhout. In: The Yeasts, a Taxonomic Study, 4th edn. (Kurtzman, C.P. and Fell, J.W., Eds.), pp. 454–573. Elsevier Science B.V., Amsterdam, The Netherlands.

[6] Kurtzman, C.P. and Fell, J.W. (1998) Summary of species characteristics. In: The Yeasts, a Taxonomic Study, 4th edn. (Kurtzman, C.P. and Fell, J.W., Eds.), pp. 915–947. Elsevier Science B.V., Amsterdam, The Netherlands.

[7] Nakase, T., Itoh, M., Takematsu, A. and Komagata, K. (1988) *Candida tanzawaensis*, a new species of yeast isolated from moss collected in Japan. Trans. Mycol. Soc. Jpn. 29, 331–338.